

APPLICANT(S): ZIV, Ilan et al.
SERIAL NO.: Not yet assigned
FILED: Herewith
Page 2

AMENDMENTS TO SPECIFICATION

In the Specification:

On page 1, immediately after the title, please insert:

PRIOR APPLICATION DATA

--This application is a National Phase application of PCT International Application No. PCT/IL2005/000055, International Filing Date: January 16, 2005, claiming priority from U.S. Provisional Patent Application Serial Number 60/536,493, entitled "PERTURBED MEMBRANE-BINDING COMPOUNDS" filed January 15, 2004, U.S. Provisional Patent Application Serial Number 60/537,289, entitled "PERTURBED MEMBRANE-BINDING COMPOUNDS" filed January 20, 2004 and U.S. Patent Application Serial Number 10/799,586, entitled "PERTURBED MEMBRANE-BINDING COMPOUNDS AND METHODS OF USING THE SAME" filed March 15, 2004, all of which are incorporated by reference in their entirety.—

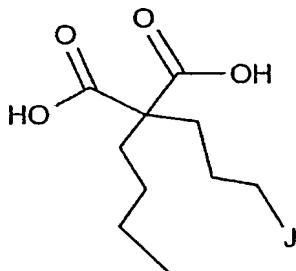
Please replace the paragraph beginning on page 3, line 27 and ending on page 4, line 2 with the following paragraph:

-- [0009] The term "*perturbed membrane-binding compound*" (PMBC) refers to a compound that ~~selective~~ **selectively** targets PNOM-cells, while binding to a lesser degree to normal cells. According to the invention, binding of the PMBC to the PNOM-cell should be a least 30% greater than its binding to the normal cell. --

Please replace the paragraph beginning on page 8, line 13 and ending on page 9, line 2 with the following paragraph:

--[0022] In yet another embodiment of the invention there is provided a compound represented by the structure as set forth in formula (VI):

APPLICANT(S): ZIV, Ilan et al.
SERIAL NO.: Not yet assigned
FILED: Herewith
Page 3



VI

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (VI) and solvates and hydrates of the salts; wherein J is selected from hydrogen, -F and -OH. In the case that J is -F, the compound is designated NST204 NST205.--

Please replace the paragraph beginning on page 13 line 7 and ending on line 12 with the following paragraph:

--[0026] In another aspect of the invention, there is provided a pharmaceutical composition for targeting of drugs to foci of apoptosis or blood clotting in a patient, wherein the patient is may be a human or non-human mammal, wherein the pharmaceutical composition comprising a compound according to the structure set forth in formulae I, II, III, IV, V, VI, VII, VIII, IX, X, XI XII, XIII, or XIV wherein the compound comprises or is being linked to a drug.--

Please replace the paragraph beginning on page 14 line 11 and ending on line 20 with the following paragraph:

--[0029] In another aspect of the invention, there is provided a method for detecting of PNOM-cells in a patient or an animal, the method comprising: (i). administering to the patient or animal a compound represented by the structure set forth

APPLICANT(S): ZIV, Ilan et al.
SERIAL NO.: Not yet assigned
FILED: Herewith
Page 4

in formulae I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, or XIV, or pharmaceutically acceptable salts, metal chelates, solvates and hydrates of the compound represented by the structure as set forth in formulae I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, or XIV, and solvates and hydrates of the salts; and (ii) imaging the examined patient or animal, so as determine the amount of the compound bound to cells, wherein a significant amount of compound bound to a cell indicates that the cell is a PNOM-cell.--

Please replace the paragraph beginning on page 14 line 21 and ending on line 26 with the following paragraph:

--[0030] In another aspect of the invention, there is provided a pharmaceutical composition for targeting of drugs to foci of apoptosis or foci or activated platelets in a blood ~~elott~~ clot in a patient or an animal, the pharmaceutical composition comprising a compound according to the structure set forth in formulae I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, or XIV, wherein the compound comprises or is being linked to a drug.--

Please replace the paragraph beginning on page 16 line 1 and ending on line 9 with the following paragraph:

-- [0031] In another embodiment, the invention provides a method of detecting cells undergoing a death process within a tumor in an examined subject, the method comprising:(i) administering a to the examined subject a compound according to the structure set forth in formulae I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, or XIV, wherein the compound comprises or is linked to a marker for imaging or a labeled metal chelate; and (ii) determining the amount of the compound bound to cells within the tumor, wherein detection of a significant amount of the compound bound to cells in the tumor indicates that these tumor cells are undergoing a death process.--

APPLICANT(S): ZIV, Ilan et al.
SERIAL NO.: Not yet assigned
FILED: Herewith
Page 5

Please replace the paragraph beginning on page 18 line 1 and ending on line 2 with the following paragraph:

-- [0043] **Figure 10 (A and B)** demonstrates autoradiography by tritium-labeled NST200 of rat renal ischemia ~~reperfusion~~ **reperfusion**; (A) damaged kidney; (B) intact kidney.--

Please replace the paragraph beginning on page 18, line 18 and ending on line 23 with the following paragraph:

-- [0047] The compounds of the invention have the advantage of being active in performing selective targeting of PNOM-cells, while also featuring a relatively low molecular weight, and a potentially favorable pharmacokinetic profile. In one embodiment of the invention, there is provided a compound which selectively targets to a PNOM cell (i.e., a PMBC) wherein the ~~wherein the~~ compound is represented by the structure set forth in formula (I): --

Please replace the paragraph beginning on page 20, line 19 and ending on page 21, line 7 with the following paragraph:

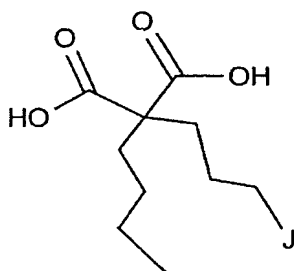
-- [0052] including pharmaceutically acceptable salts, hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (II) and solvates and hydrates of the salts; wherein R represents hydrogen or C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂, C₁₃, C₁₄, C₁₅, C₁₆, linear or branched alkyl, linear or branched hydroxy-alkyl, linear or branched fluoro-alkyl, aryl or heteroaryl composed of one or two rings, or combinations thereof; n and m each stands for an integer of 0, 1, 2, 3 or 4; n and m may be same or different; M is selected from null, hydrogen, -O-, -S-, and -N(U), wherein U stands for a null, hydrogen, C₁, C₂, C₃, or C₄ alkyl; D is hydrogen or a marker for diagnostics, selected from a marker for imaging such ¹⁸F, or a labeled metal chelate; the marker for imaging may be detected by color, fluorescence, x-ray, CT scan, magnetic resonance imaging (MRI) or radio-isotope scan such as single photon

APPLICANT(S): ZIV, Ilan et al.
SERIAL NO.: Not yet assigned
FILED: Herewith
Page 6

emission tomography (SPECT) or positron emission tomography (PET). Alternatively, D is a drug to be targeted to the PNOM cells, as ~~define~~ defined above. --

Please replace the paragraph beginning on page 22, line 15 and ending on page 23, line 6 with the following paragraph:

-- [0057] In yet another embodiment of the invention there is provided a compound represented by the structure as set forth in formula (VI):



VI

including pharmaceutically acceptable salts hydrates, solvates and metal chelates of the compound represented by the structure as set forth in formula (VI) and solvates and hydrates of the salts; wherein J is selected from hydrogen, -F and -OH. In the case that J is -F, the compound is designated ~~NST204~~ NST205. --

Please replace the paragraph beginning on page 28, line 28 and ending on page 29, line 9 with the following paragraph:

-- [0065] According to another aspect of the invention, there is provided a method for detecting of PNOM-cells in a patient or an animal, the method comprising: (i) administering to the patient or animal a compound ~~a compound~~ represented by the structure set forth in formulae I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, or XIV, wherein the compound comprises a marker for imaging, such as ^{18}F or pharmaceutically acceptable salts, metal chelates, solvates and hydrates of the compound represented by

APPLICANT(S): ZIV, Ilan et al.
SERIAL NO.: Not yet assigned
FILED: Herewith
Page 7

the structure as set forth in formulae I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, or XIV, and solvates and hydrates of the salts; and (ii) imaging the examined patient or animal, so as to determine the amount of compound bound to cells, wherein detection of a significant amount of compound bound to cells indicates that these cells are PNOM-cells. --

Please replace the paragraph beginning on page 30 line 12 and ending on line 21 with the following paragraph:

-- [0068] In a non limiting hypothesis of the mode of action of the NST-ML-Action Motif, it comprises a *switch moiety*, activated selectively upon its approaching a membrane which features the above characteristics, i.e., the plasma membrane of an apoptotic cell (Figure 1). The Action Motif is soluble in physiological pH, due to its having two negatively-charged carboxylate groups (pKa of alkylmalonate is about 5.6 and 2.8), thus having mostly a formal charge of -2 in physiological conditions. However, upon approaching the apoptotic membrane, due to the more acidic surface, and due to the reduction in the dielectric constant of the interfacial environment, which acts to elevate pKa values of the carboxyl groups, a proton is being captured by the malonate moiety moiety.--

Please replace the paragraph beginning on page 30, line 22 and ending on page 31, line 13 with the following paragraph:

-- [0069] The capture of the proton by the malonate group neutralizes one of the negative charges, thus rendering the molecule more hydrophobic, with an overall charge of -1. Moreover, the capture of the proton further leads to a very unique situation, which includes the following:

- (i). An acid-anion pair is formed, wherein an exceptionally strong hydrogen bond is formed between the protonated and unprotonated

carboxyl groups. This hydrogen bond is strong, symmetrical and stabilized by resonance and ~~tautamerization~~ tautomerization.

(ii). This leads to distribution of the negative charge over the four carboxyl atoms, i.e., its being partially delocalized.

(iii). The strong acid-anion hydrogen bond rigidifies the molecule, creating a bulky, rigid, flat, six-membered ring, bearing a partially-delocalized negative charge, and comprising pi-electron clouds over the carboxyl ~~double~~ double bonds. Such an element can undergo a relatively favorable penetration into the membrane interface, according to a non-limiting hypothesis of the mechanism of action of the compounds of the invention. However, its bulky, rigid structure directs its binding selectively to loosely packed membranes, i.e., apoptotic ~~membranes~~ membranes, while precluding binding to highly-packed membranes, such as the plasma membranes of healthy cells. These steric features therefore promote selectivity in binding to the apoptotic membranes. --

Please replace the paragraph beginning on page 31 line 22 and ending on line 26 with the following paragraph:

--[0071] The penetration of the protonated malonate moiety into the membrane interface and the stabilization of its binding in the interface, allow the alkyl chain R to traverse the membrane interface and to reach its optimal binding environment, i.e., the membrane hydrocarbon core, whereupon it will further contribute through ~~hydrophobic~~ hydrophobic interactions to the free energy gain of compound binding. --

Please replace the paragraph beginning on page 31, line 27 and ending on page 32, line 4 with the following paragraph:

-- [0072] The NST-ML-Action Motif is being utilized for useful diagnostic or therapeutic purposes, through its binding to a marker for imaging or a therapeutic drug (moiety D in Formula I) through a hydrocarbon linker $[(CH_2)_m]$ of Formulae I or 2]. The

APPLICANT(S): ZIV, Ilan et al.
SERIAL NO.: Not yet assigned
FILED: Herewith
Page 9

NST-ML-Action Motif according to this approach acts as a targeting ~~moeity~~-moiety , allowing selective targeting of the marker for imaging or the drug attached to it to cells and tissues inflicted by cell death, ~~paricularly~~ particularly apoptosis, or tissues inflicted by platelet activation and thrombosis. --

Please replace the paragraph beginning on page 32 line 5 and ending on line 25 with the following paragraph:

-- [0073] Figure 1 demonstrates NST200 (Formula IV), and describes the three stages of its approach and binding to the PNOM membrane in physiological conditions:

- A: The compound is in an aqueous solution, thus both carboxyl groups are deprotonated, i.e., negatively charged, and the compound is highly soluble.
- B: Upon approaching the negatively-charged apoptotic membrane, the compound acquires a proton. An anion-acid dimer is formed, thus creating a stable six-membered, resonance-stabilized ring, which penetrates the membrane interface. The bulky, rigid ring structure assists in selectivity, since its steric features favor binding to the more loosely-packed plasma membrane of the apoptotic cell, rather than binding to the well-packed plasma membrane of the healthy cell.
- C: Upon penetration of the compound into the membrane interface, it is subjected to the interfacial network of hydrogen bonds, and to the augmented interfacial proton currents encountered in the interface of the apoptotic membrane. The resultant protonation and hydrogen bonding further acts to stabilize the binding of the compound to the interface (arrows). Such penetration further allows the alkyl chain to reach its optimal position within the membrane, thus further contributing to the binding energy, through formation of hydrophobic interactions with the membrane hydrocarbon core. --

APPLICANT(S): ZIV, Ilan et al.
SERIAL NO.: Not yet assigned
FILED: Herewith
Page 10

Please replace line 27 on page 43 with the following:

-- Synthesis of NST-ML-F-4 (2-butyl-2(3-fluoropropyl)-malonic acid, **NST205**); (Scheme 1):--

Please replace lines 7 and 8 on page 45 with the following:

-- Synthesis of NST-ML-F: 2-methyl-2(3-~~fluorobutyl~~-~~fluoropropyl~~)-malonic acid; **NST201** (Scheme 2):--

Please replace the paragraph beginning on line 4 and ending on line 7 on page 53 with the following paragraph:

-- The wide spectrum of ^3H -NST200 accumulated values, reflects the individual response of different tumors to the anti cancer treatment. The above experiment clearly shows that ^3H -NST200 can serve for detecting carcinoma and ~~the~~ for detecting the effect of cytotoxic drugs on the carcinoma cells.--

Please replace the paragraph beginning on line 10 and ending on line 13 on page 54 with the following paragraph:

-- Two days after the second doxorubicin injection, mice were injected i.v. with 10 μCi of ^3H -NST200 in a volume of 0.2 ml saline. Four hours following ^3H -NST200 injection, mice were sacrificed by penthal overdosing. Tumors were collected in ependorff tubes, weighted and ~~freezed~~ **frozen** in -20°C .--

Please replace the paragraph beginning on line 2 and ending on line 10 on page 55 with the following paragraph:

-- During the treatment with doxorubicin (lasting 5 days), the tumor mass was not reduced (see Figure 8A). In contrast, the tumor ~~continue~~ **continued** to grow, exhibiting a 50% increase in their volume, as compared to the control non-

APPLICANT(S): ZIV, Ilan et al.
SERIAL NO.: Not yet assigned
FILED: Herewith
Page 11

treated tumors, that were collected 5 days earlier than the treated tumors. This increase was found to be non-significant. While such non-significant change in tumor volume occurred after 2 doses of doxorubicin treatment, a dramatic increase, by 18.5 fold in ^3H -NST200 uptake was detected, indicating that ^3H -NST200 is a sensitive tool, capable of sensing cell death within the tumor even in cases where no shrinkage of tumor mass is detected (see Figure 8B).--

Please replace the paragraph beginning on line 21 and ending on line 26 on page 56 with the following paragraph:

-- As can be seen from Figure 9, which shows accumulation of ^3H -NST 200 (a) and H&E staining (b), autoradiographic image analysis revealed the specificity of targeting the injury by ^3H -NST200 within the regions of ~~apoptotic~~apoptotic /necrotic cell death. The results were further confirmed by H& E staining. Accordingly, ^3H -NST200 can be used as a marker for brain apoptotic damage in autoradiographic image analysis.--